Reference: *Biol. Bull.*, 138: 77–87. (February, 1970)

THE ROLE OF ILLUMINATION AND TEMPERATURE IN THE CONTROL OF SEXUAL REPRODUCTION IN THE PLANARIAN *DUGESIA TIGRINA* (GIRARD)

C. VOWINCKEL

Department of Zoology, McGill University, Montreal, Canada

It is well established that the timing of reproductive activity in higher animals such as vertebrates and arthropods is dependent on environmental stimuli which set in train various physiological mechanisms. In lower organisms little exact knowledge is so far available on the nature of such controlling stimuli.

Planarians belong among the most primitive Bilateria. In spite of their apparent simplicity they have envolved morphologically complex organs of reproduction and, in many species, a seasonal alternation of reproduction between sexual and asexual pathways.

Such a species is *Dugesia tigrina* (Girard). In the St. Lawrence River it develops its reproductive organs in fall, lays cocoons in early summer and reproduces asexually by fission for the rest of the warm season. The object of the present enquiry is the nature of the stimulus that will induce the change from asexual reproduction to the development of sex organs in late summer in this species.

Previous experimentation on the action of environmental agents on the induction of sexual development by Castle (1928), and Hyman (1934ab) emphasized the importance, for planarians, of low water temperatures, which appears well established. Vowinckel (1970) showed that exposure to such temperatures stimulates an increase in germ cells associated in testicular primordia and that this activity is transmittable via homogenates.

In view, however, of the widespread control exerted by day length over the seasonal reproductive cycle of many animal groups it appears conceivable that sexual reproduction in planarians is regulated not only by temperature but controlled additionally by photoperiod. The work reported below puts this concept to a test.

Materials and Methods

Dugesia tigrina was collected from its natural habitat in late summer and maintained at 27° C till November. During this time sexual structures already acquired under natural conditions disappeared slowly and the population finally returned to asexual reproduction. Fission products were not removed and when experimentation began the population consisted partly of individuals grown up under natural habitat conditions and partly of individuals reared under incubator conditions. This population was used for experiment 1 (series A-D).

The population was maintained in white enamel pans in a 9 cubic foot incubator at 27° C and a constant photoperiod of LD 16:8 (ON from 0600 to 2200). Nine inch fluorescent tubes served as light sources. They were controlled by automatic clock switches and were reset daily in series which experienced photoperiod varia-

tions. In the first experiment discussed below, series A and C (Figure I, Table I), light conditions were varied as follows: from 16 hours daylight the photoperiod was curtailed by 7–8 minutes every morning and evening so that each successive day was 15 minutes shorter. In addition the light intensity was reduced in 3 steps from 1500 lux to 750, 280, and 175 lux approximately by enclosing the fluorescent light with sheets of paper. When the photoperiod had reached LD 8:16 the whole process was reversed until LD 16:8 and full intensity were reestablished.

In the second experiment, series E (Table II), day length decrease followed the same regime, light intensity was decreased in 9 small steps from 1500 lux to 200 lux approximately, using a shutter which gradually closed over the fluorescent light.

The temperature was decreased by approximately 1° C every second day in the first experiment. This took the planarians from 27° C to 12° C in 30 days. The temperature regime for experiment 2 is given in Table II.

One individual from this population, after laying cocoons in the laboratory, was used to rear a clone from its fission products. This clone was maintained also at LD 16:8 but at 24° C since this temperature more closely corresponds to summer conditions in the population's natural habitat. This clone supplied the material for the second experiment (series E-G).

All animals were fed veal liver 3 times a week and their culture water replaced from bottles kept within the incubator. Unchlorinated river water was used throughout.

The experiments reported below were conducted in four 4 cubic foot incubators, series A, B, C and D concurrently and series E, F and G later. Experimental animals were maintained in 9 × 5 cm glass petri dishes with black lids.

Evaluation methods: The degree of sexual development was determined histologically. Animals were fixed in Bouin's fixative, wax-embedded under vacuum, serial sections cut a 16 micra and stained with aldehyde fuchsin using phloxin as counter stain.

A scale of 10 stages was developed for evaluation by Vowinckel (1968) and is given below in abridged form:

stage 1: 2–5 cells typical size of testicular primordia
stage 2: 6–10 cells typical size of testicular primordia
stage 3: 11–20 cells typical size of testicular primordia
stage 4: maturation divisions begin in most testicular follicles, testicular lumen forms
stage 5: most testicular follicles contain mature sperm
stage 6: future copulatory complex indicated by a round cavity
stage 7: penis mound recognizable within this cavity
stage 8: female antrum has arisen posterior to male antrum
stage 9: bursal stalk and bursa have appeared, gonopore opens
stage 10: ovovitelline duct and sperm duct have connected with copulatory complex

Results

Four groups of 45 planarians each were randomly selected from the population collected in the natural habitat. All showed signs of recent fission. They were

exposed to 4 different temperature and light regimens. In one group both factors were varied, in two groups only one factor was varied and in the last group both factors were held constant. The variations all followed the same scheme: the factor was reduced for 30 days at a constant daily rate and then the trend was reversed and the factor increased again at the same rate.

When temperature or light conditions were not varied they were held at 27° C and LD 16:8, the animals therefore experienced no change in these parameters from the conditions in the stock incubator.

In the four series the combinations of light and temperature regimens were:

A: light varied, temperature varied.
B: light varied, temperature not varied.
C: light not varied, temperature varied.
D: light not varied, temperature not varied.

Animals were sacrificed at increasing intervals: twice daily (first 3 days), daily (next 3 days) every second day (for 38 days) every third day (for 24 days) and all remaining individuals on day 72. Serial sections were then scanned for the degree of sexual development.

The results of this evaluation are given in Figure 1. Sexual development resulted when either or both factors were varied (series A, B, C), no sexual development resulted if both factors were held constant (series D). There are some differences between those series that responded positively. When both light and temperature were varied (series A) 9 individuals reached marked testicular development (stage 3 or more). When only light was varied 6 individuals reached stage 3 or more and if temperature alone was varied 11 individuals reached or surpassed stage 3.

In order to evaluate further the differences between the series a count was made of all sections of testicular follicles containing spermatids and/or mature sperm in each worm. This count is given in Table I. It shows clearly that in series A (both factors varied) no individual reached testicular maturation. In contrast photoperiod variations alone (series B) caused fewer individuals to respond but testis maturation was reached, at least in some follicles, in all animals that responded.

During the experiment all fission products were counted and removed. The count was checked against the number of worms which had fissioned. The results are given in Figure 2 where the count is expressed as per cent of the existing population and summed over successive periods of 5 days. The fission rate demonstrates the basic difference between the animals which only received illumination variations (B) and those which received temperature variations (A and C). The series which received changes in daylight only continued to fission throughout the duration of the experiment as did the control series D. Both series that were exposed to decreases in temperature on the other hand ceased to fission until conditions again approached those of the control series.

Since series B (illumination variations only) continues to fission no stage higher than stage 5 can be reached by this group. The developing copulatory complex is always lost with the fission product. Normally testicular development continues after stage 5 by growth of follicles and by maturation of late developing

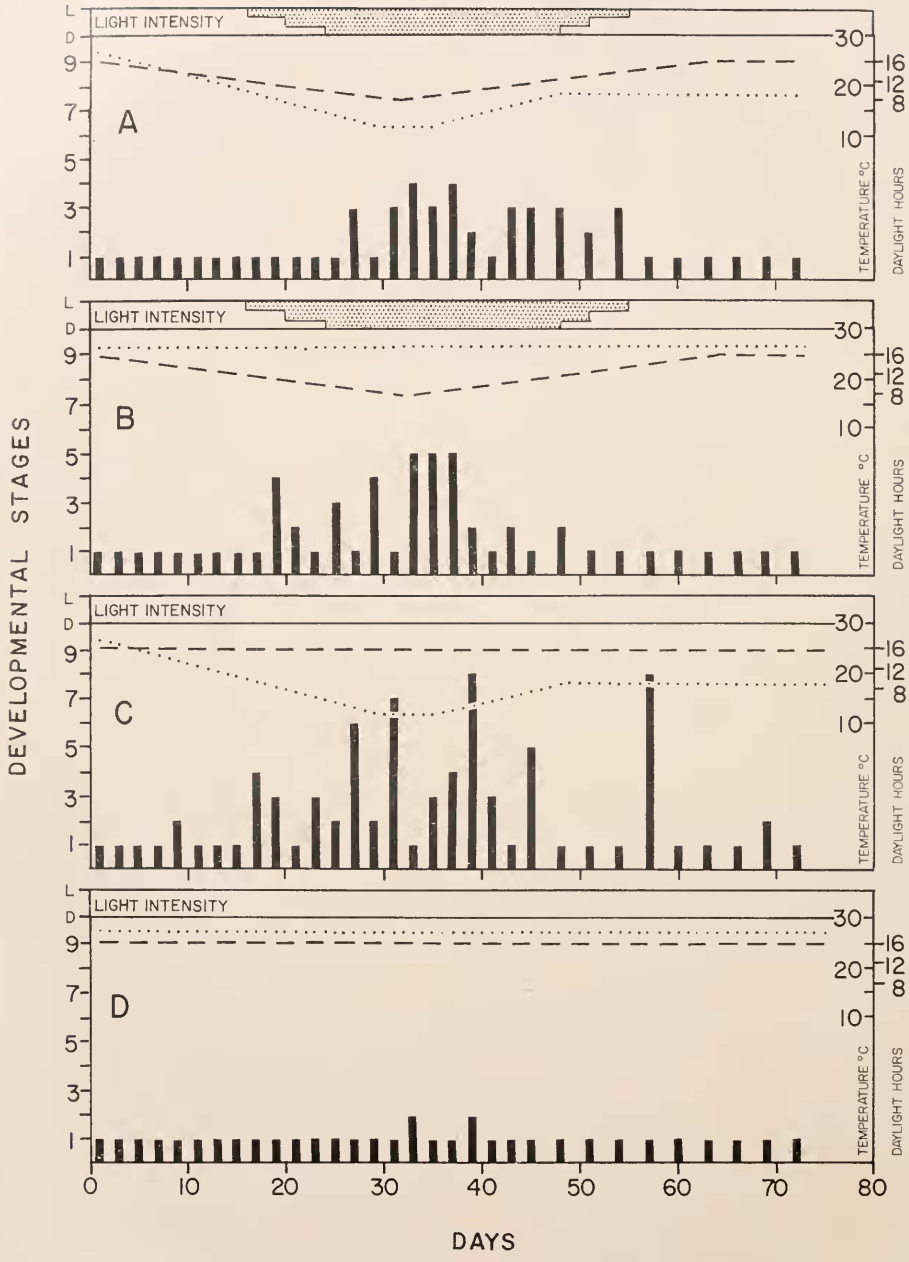

FIGURE 1. Degree of sexual development of the natural population when daylight and/or temperature are progressively decreased. Each column represents one individual; - - - - photoperiod, ... temperature. Not all individuals on days 1–6 and after day 69 are entered. Individuals that were omitted all reached stage 1 only.

TABLE 1

Number of cross sections of testis follicles per worm containing spermatids and/or mature sperm. Total number of worms containing sperm in series A = 0, B = 7, C = 6, D = 0. Population collected from natural habitat

Series	A	B	C	D	Number of animals/series
	Light varied temp. varied	Light varied temp. const.	Light const. temp. varied	Light const. temp. const.	
Day					
1–15	0	0	0	0	14
17	0	0	95	0	1
19	0	2	0	0	1
21	0	0	0	0	1
23	0	0	0	0	1
25	0	25	0	0	1
27	0	0	91	0	1
29	0	3	0	0	1
31	0	5	592	0	1
33	0	402	0	0	1
35	0	108	0	0	1
37	0	364	0	0	1
39	0	0	84	0	1
41	0	0	0	0	1
43	0	0	0	0	1
45	0	0	37	0	1
48	0	0	0	0	1
51	0	0	0	0	1
54	0	0	0	0	1
57	0	0	804	0	1
60–72	0	0	0	0	9–12
	0	909	1703	0	42–45

follicles. For this reason the number of follicular sections containing sperm which are typical for stages 6–8 in series C (temperature variations only) are in series B found with stage 5, as an abridged rearrangement of Table I shows:

	Stage	Sections c sperm	Day		Stage	Sections c sperm	Day
Series B	5	402	33	Series C	5	37	45
(light only	5	108	35	(temperature	6	91	27
varied)	5	364	37	only varied)	7	541	31
					8	84	39
					8	804	57

Three series of this experiment (B, C, D) were repeated with incubator reared, cloned animals which descended from an individual which had previously laid viable cocoons in the laboratory. These 3 series were conducted as follows:

Series E: light conditions varied, temperature maintained constant.
Series F: light conditions maintained constant at full intensity; temperature decreased.
Series G: light conditions maintained constant at full intensity; temperature maintained constant at 24° C.

TABLE II

Degree of sexual development of incubator reared individuals exposed to gradually reduced illumination and temperature conditions. Stage = degree of development reached by individual fixed on date indicated. For explanation of stages see text. Series G: neither temperature nor light varied, series E: light varied only, series F: temperature varied only

Day	Series G				E					F			
	Number of fission products	Stage	Temperature (Centigrade)	Daylength (hr min)	Number of fission products	Stage	Light intensity reduced	Temperature (Centigrade)	Daylength (hr min)	Number of fission products	Stage	Temperature (Centigrade)	Daylength (hr min)
1			23.2	16.00				23.5	16.00			23.5	16.00
2	2		22.8	16.00	13			23.5	15.52	0		23.0	16.00
3	5		23.3	16.00	2		x	23.7	15.37	0	2	23.1	16.00
4	3		23.3	16.00	6			23.7	15.22	7		24.1	16.00
5	3		23.3	16.00	1	2		23.6	15.07	1		24.5	16.00
6	9		23.4	16.00	0			23.5	14.52	4		23.1	16.00
7	1		23.4	16.00	4		x	23.2	14.37	0	2	22.6	16.00
8	4	3	23.3	16.00	3			23.2	14.22	0		22.6	16.00
9	0		23.2	16.00	3	3		23.3	14.07	3		22.0	16.00
10	2		23.3	16.00	5			23.5	13.52	1		21.6	16.00
11	1		23.9	16.00	4		x	23.5	13.37	1	2	21.4	16.00
12	1	2	23.9	16.00	4			23.3	13.22	1		21.0	16.00
13	4		23.4	16.00	0	2		22.9	13.07	2		20.2	16.00
14	4		23.5	16.00	2			23.6	12.52	0		20.2	16.00
15	6		23.5	16.00	4		x	23.1	12.37	0	2	19.7	16.00
16	5	2	23.7	16.00	2			23.5	12.22	1		19.7	16.00
17	3		23.6	16.00	3	2		23.0	12.07	0		19.1	16.00
18	1		23.3	16.00	4			23.7	11.52	0		19.2	16.00
19	1		23.5	16.00	1		x	23.6	11.37	0	2	18.2	16.00
20	3	2	23.3	16.00	1			22.9	11.22	0		18.2	16.00

TABLE II—(*Continued*)

Day	Series G				E					F			
	Number of fission products	Stage	Temperature (Centigrade)	Daylength (hr min)	Number of fission products	Stage	Light intensity reduced	Temperature (Centigrade)	Daylength (hr min)	Number of fission products	Stage	Temperature (Centigrade)	Daylength (hr min)
21	2		23.6	16.00	3	2		23.3	11.07	0		17.6	16.00
22	5		23.5	16.00	5			23.2	10.52	0		17.7	16.00
23	2		23.5	16.00	1		x	23.3	10.37	0	2	17.3	16.00
24	3	2	23.3	16.00	1			23.7	10.22	0		17.3	16.00
25	3		23.4	16.00	1	2		23.7	10.07	0		17.0	16.00
26	1		23.5	16.00	4			23.8	9.52	0		16.8	16.00
27	4		23.3	16.00	3		x	22.8	9.37	0	3	16.3	16.00
28	3	2	23.5	16.00	7			23.5	9.22	0		16.2	16.00
29	9		23.4	16.00	1	2		23.8	9.07	0		15.6	16.00
30	0		23.4	16.00	1			24.1	8.52	0		15.8	16.00
31	3		23.3	16.00	2		x	22.8	8.37	0	3	14.8	16.00
32	1	2	23.6	16.00	0			23.3	8.22	0		15.0	16.00
33	0		23.5	16.00	3	2		23.2	8.07	0		14.0	16.00
34					2			23.1	7.52	0		14.1	16.00
35					1		x	23.3	7.37	0	3	14.1	16.00
36					8			22.8	7.22	0		14.3	16.00
37					1	2		22.9	7.07	0		13.8	16.00
38								23.3		0		13.8	16.00
39								23.3		0	3	14.0	16.00
40								23.2		0		13.2	16.00
41								23.2		0		13.1	16.00
42										0		12.8	16.00
43										0	2	12.8	16.00

Animals were sacrificed every fourth day and evaluation was done by the same method as before.

The results are given in Table II and show plainly that in spite of the fact that conditions are closely similar to series B-D no sexual development resulted beyond stage 3 in the cold exposed series (F) and that the changes in photoperiod resulted not even in a size increase of testicular primordia. The fission rate responds in a normal manner. In series F fission ceases as the temperature drops below 20° C and persists in series E and G where the temperature remains at 24° C.

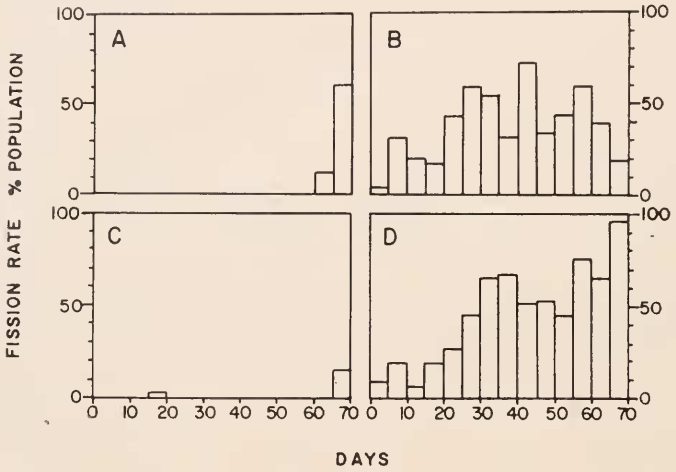

FIGURE 2. Fission rates in the four series of experiment 1 (natural population) expressed as per cent of population and summed over 5 day periods; series A: light and temperature varied, series B; light only varied, series C: temperature only varied, series D: neither light nor temperature varied.

Numerous further attempts with various temperature regimen, *e.g.*, fast drops in temperature, diurnal temperature variations with the daily mean temperature decreasing slowly and a succession of small stepwise decreases have all been equally unsuccessful in inducing sexual development in the incubator reared material.

DISCUSSION

The results of our experimentation indicate that changes in photoperiod as well as temperature can induce sexual development. The sensitivity of planarians to light is well established. Recently May and Birukow (1966) found that *D. gonocephala* has a variable preference for light intensities if given a choice. This preference varies in correlation with the phases of the moon during the synodial month. Ude (1964) correlated natural and reversed day and night rhythms with neurosecretory activity in *Dendrocoelum lacteum* and found that animals fixed during the light period differed from those fixed during the dark period by the number of cells showing histological signs of neurosecretory activity. Both Ude (1964) and Lender (1964) found that neurosecretory activity—as judged from staining affinities of nerve cells—is at a peak during the time when sex organs are

developing in planarians. Our finding that a reduction in period and intensity of illumination can artificially induce sexual development therefore agrees well with their results.

The reason why in series A the combination of the two factors acts to retard sexual development (Table I) as compared to series B and C is not immediately obvious. A comparison was made with the natural habitat conditions (Vowinckel 1968). In 1967 daily temperature maxima were recorded at the place of collection of the population. On August 27 some members of the natural population already contained mature sperm in their testes. The highest water temperature of the summer, 25° C, was recorded on August 26 and the minimum on the night before for the first time fell below 20° C. The water temperatures at the time of induction of sexual development, therefore, are those of high summer. Daily illumination, on the other hand, at this time has been steadily reduced for two months, both in intensity and photoperiod. It appears therefore possible that under natural conditions changes in illumination represent the inducing stimulus while a decrease in temperature stimulates sexual development later in the season and inhibits fission.

It is known that temperature and photoperiod, within limits, interact, and can to some extent replace each other, as releasing stimuli in the induction of sexual development of organisms. Recent examples are given by Minis and Pittendrigh (1968) and Lees (1959, 1963).

In series B fission continues although differentiation towards sexual reproduction has proceeded to a point where all testis follicles contain mature sperm. This seems to indicate that two different mechanisms are involved in the control of asexual and sexual reproduction. If only one factor ruled both phenomena, acting as stimulant for one pathway and as inhibitor for the other, such overlap should not occur.

Brown (1965) and Brown and Park (1965) have brought evidence that changes in the horizontal magnetic field can be correlated with changes in the behavior of *D. dorotocephala*. They suggest that the regular cyclic variations of this field may be responsible for the timing of physiological rhythms. Unfortunately, under normal experimental conditions geomagnetic variations are not easily controlled. Using a gauss meter (Bell, model 620) we found that the earth's field was reduced to approximately 0.06 *g* inside the cabinet of our incubators. Since all four incubators were identical in make and were run concurrently on the same table it should be expected that any variations of the earth"s field had the same effect in all incubators. No change in the magnetic vector could be detected when the fluorescent lights were switched on or off. The sexual development resulting in series B should, therefore, be correlated with the changes in illumination. The cooling motors, on the other hand, gave a brief surge of 0.01 *g* whenever they were activated or deactivated. Series A and C, therefore, differed from series B and D not only in temperature but also in the properties of the magnetic vector since in the latter two series the cooling motors did not activate, 27° C being above room temperature. However, it is felt that in view of the well established influence of water temperature on planarian sexual development it would be precipitous to assign an inductive role to a factor which must have shown variations very different from the pattern of the natural environment.

Kenk (1940) suggested that planarians have an inherent rhythm of sexual reproduction, independent of environmental control. This suggestion was accepted by Goldsmith (1942) and Dahm (1958) but rejected by Hyman (1943b) who argued that it is possible to induce sexuality arhythmically by using temperature treatments. Our incubator reared descendents of a parent which had proven its ability to lay viable cocoons have never reproduced sexually although the clone is maintained now for over 3 years. From this evidence we deduce that sexual reproduction in *D. tigrina* cannot be due to an inherent rhythm but rather depends on environmental stimuli which under these special maintenance conditions are absent.

When mature, our incubator reared individuals neither respond to reductions in photoperiod nor to reductions in temperature closely similar to those eliciting positive results in individuals that grew up under natural conditions. The altered maintenance temperature could hardly be the cause for this difference in response since it resembles more closely the natural conditions. Our incubator reared animals appear healthy in every respect, have a normal fission rate (approximately once every 8 days) and respond to a drop in temperature with an increase in the number of germ cells associated in testicular primordia (Vowinckel, 1970) which suggests that they have not lost their ability to respond. Their presence in small numbers in the mixed population may explain why some individuals in series A-C fail to respond to the stimuli.

Since nature animals collected from the natural habitat in late summer regularly react to illumination or temperature reductions with sexual development (Hyman 1943a, Vowinckel 1968) an occurrence prior to collection must have "triggered" them to respond to later stimulation. The "setting of the trigger" may be possible only during a short period in the development of the planarian. At present we are investigating this possibility.

This work was supported by a McConnell Fellowship of McGill University to the author and a National Research Council of Canada grant to Professor Joan Marsden in whose laboratory the experiments were performed and to whom the author is indebted for valuable advice and criticism. Part of the work reported here was included in a Ph.D. thesis submitted to McGill University.

Summary

1. Planarians were collected from the natural habitat in late summer and exposed to gradual reductions in illumination and/or temperature.

2. They responded to variations of either or both factors with sexual development while the control group under constant temperature and light conditions did not enter sexual development.

3. Asexual reproduction (fission) was inhibited by low temperatures and apparently not influenced by changes in illumination.

4. Incubator reared, cloned descendents of an individual which had previously laid viable cocoons did not respond to the same treatment.

5. The results are discussed and the importance of the difference between the two populations' response is interpreted.

LITERATURE CITED

Brown, F. A., Jr., 1965a. Duration of an after-effect in planarians following a reversed horizontal magnetic field. *Biol. Bull.*, **128**: 347–355.

Brown, F. A., Jr., and Y. H. Park, 1965b. Phase shifting a lunar rhythm in planarians by altering the horizontal magnetic vector. *Biol. Bull.*, **129**: 79–86.

Castle, W. A., 1928. An experimental and histological study of the life cycle of *Planaria velata*. *J. Exp. Zool.*, **51**: 417–483.

Dahm, A. G., 1958. *Taxonomy and Ecology of Five Species Groups in the Family Planariidae (Turbellaria, Tricladida, Paludicola)*. Nya Lintografen 12158, Malmø, 241 pp.

Goldsmith, E. D., 1942. Sexuality in *Dugesia tigrina*. *Nature*, **150**: 351.

Hyman, L. H., 1943a. Environmental control of sexual reproduction in flatworms. *Anat. Rec.*, **81**: 108.

Hyman, L. H., 1943b. Water content of medusae, sexuality in a planarian. *Nature*, **151**: 140.

Kenk, R., 1940. The reproduction of *Dugesia tigrina* (Girard). *Amer. Natur.*, **74**: 471–475.

Lees, A. D., 1959. The role of photoperiod and temperature in the determination of parthenogenetic and sexual forms in the aphid *Megoura viciae* Buckton—I The influence of these factors on apterous virginoparae and their progeny. *J. Insect Physiol.*, **3**: 92–117.

Lees, A. D., 1963. The role of photoperiod and temperature in the determination of parthenogenetic and sexual forms in the aphid *Megoura viciae* Buckton—III Further properties of the maternal switching mechanism in the apterous aphids. *J. Insect Physiol.*, **9**: 153–164.

Lender, T., 1964. Mise en évidence et rôles de la neurosécrétion chez les planaires d'eau douce (Tourbellariés, Triclades). *Ann. Endocrinol.*, **25**(5): 61–65.

May, E., and G. Birukow, 1966. Lunarperiodische Schwankungen des Lichtpraeferendums bei der Bachplanarie. *Naturwissenschaften*, **53**: 182.

Minis, D. H., and C. S. Pittendrigh, 1968. Circadian oscillation controlling hatching: Its ontogeny during embryogenesis of a moth. *Science*, **159**: 534–536.

Ude, J., 1964. Untersuchungen zur Neurosekretion bei *Dendrocoelum lacteum*, Oerst. (Platyhelm. Turbell.). *Z. Wiss. Zool.*, **170**: 223–255.

Vowinckel, C., 1968. The induction of sexual development in the planarian *Dugesia tigrina* (Girard), *Doctoral dissertation, McGill University*, Montreal, 104 pp.

Vowinckel, C., 1970. The stimulation of germ cell proliferation in *Dugesia tigrina* (Girard), *J. Embryol. Exp. Morphol.*, in press.